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Bone sialoprotein in serum of patients with malignant bone diseases

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Bone sialoprotein (BS), a protein synthesized by osteoblasts and osteoclasts and highly modified posttranslationally, constitutes a predominant fraction of the noncollagenous organic matrix in human bone. We report an assessment of serum concentrations of BS in patients with malignant bone diseases. In patients with bone metastases (according to scintigraphic criteria), serum BS concentrations were greater than in patients without bone metastases ($P < 0.05$). However, ROC curve analysis revealed that serum BS was inferior to serum bone alkaline phosphatase in discriminating between patients with and without bone metastases. Patients with bone metastases showed a weak correlation between serum BS concentrations and bone formation markers. Only "traditional" markers of bone formation—but not BS—were correlated with urinary deoxypyridinoline ($P < 0.01$). Liver and kidney dysfunction had no significant influence on BS values in these patients (as assessed by analysis of variance; $P > 0.05$). In multiple myeloma patients treated with corticosteroids and bisphosphonates, BS concentrations were lower than in tumor patients without bone metastases ($P < 0.001$), and the correlation between BS concentrations and the number of bisphosphonate courses applied was significant ($r = -0.578$; $P < 0.05$). In postmenopausal women, serum BS concentrations averaged 142% greater than in premenopausal women. Further studies should be done, therefore, to elucidate whether serum BS is able to predict high bone turnover after menopause.

INDEXING TERMS: alkaline phosphatase • carboxyl-terminal propeptide • deoxypyridinoline • urine • calcium • bone metastases • multiple myeloma • menopausal status • radioimmunoassay

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Bone sialoprotein, a protein that is highly modified posttranslationally, constitutes a predominant fraction of the noncollagenous organic matrix in human bone *[1]*. Synthesized by osteoblasts *[1]* and osteoclasts *[2]*, it has a protein core of M_r 33 600. In contrast to other phosphorylated glycoproteins of bone matrix (e.g., osteonectin), bone sialoprotein is relatively restricted to bone. It stimulates hydroxyapatite formation in vitro *[3, 4]* and contains an RGD integrin binding sequence; possibly, therefore, it acts as a cell adhesion molecule, allowing cells to attach to the extracellular matrix *[1]*. Synthesis of bone sialoprotein in cultured osteoblastic cells is inhibited by calcitriol but stimulated by dexamethasone *[5]*. To our knowledge, only preliminary results are available concerning assessment (with an ELISA) of the serum concentrations of this putative marker of bone turnover: (*a*) In serum of patients with early rheumatoid arthritis, high concentrations of bone sialoprotein have been demonstrated *[6]*, and (*b*) in rheumatoid arthritis patients with advanced joint destruction, synovial fluid concentrations of the protein are higher than in patients with well-preserved joints, possibly reflecting enrichment of bone sialoprotein in the cartilage–bone interphase *[7]*.

Here we report the use of an RIA to determine the serum concentrations of bone sialoprotein in patients with different malignant bone diseases. We also compared the values obtained with those of established markers of bone turnover: bone alkaline phosphatase, carboxyl-terminal propeptide of procollagen type I, urinary deoxypyridinoline, and urinary calcium.

Materials and Methods

REFERENCE POPULATIONS AND PATIENTS

We analyzed serum and urine samples obtained from several groups of subjects. Group A was 66 apparently healthy persons: 30 men and 36 women, ages 33 ± 9 years [mean ± SD; ranges 21–49 years for the men, 20–49 years for the women (not significantly different: $P > 0.1$)]. None had a history of serious disease or was taking drugs known to affect bone metabolism, and all of the women had a history of regular menses.

Group B comprised 18 postmenopausal women, ages 58 ± 6 years (range 51–75 years; years since onset of menopause: 8 ± 5), and 18 age-matched men, ages 61 ± 5 years (range 55–74 years), with no history of serious disease or drug administration known to influence bone metabolism.

Group C was a consecutive series of 77 patients—29 men and 48 women, ages 57 ± 11 years (range 27–78 years)—with various malignant tumors who were routinely examined for the presence of bone metastases by ^{99m}Tc-methylene bisphosphonate bone scintigraphy in the Department for Nuclear Medicine of our hospital. According to the results of bone scintigraphy, the patients were classified as follows: (*a*) 55 patients showed either no pathological findings or only pathological findings that could be attributed to benign diseases (e.g., osteoarthritis): patients without bone metastases; and (*b*) 22 patients had scintigraphic evidence of bone metastases: patients with bone metastases. The latter patients were diagnosed with cancers of the breast (n = 27), lung (n = 13), prostate (n = 3), kidney (n = 2), thyroid glands (n = 2), esophagus (n = 3), uterus (n = 2), colon (n = 4), pharynx (n = 1), tongue (n = 1), stomach (n = 1), or tonsils (n = 1) or with malignant melanoma (n = 11), lymphoma (n = 4), or Hodgkin's disease (n = 2). Thirteen of the patients in the "no bone metastases" group had breast cancer. None of the patients in either group showed hypercalcemia.

Group D consisted of 16 patients—13 men and 3 women, ages 62 ± 6 years (range 47–67 years)—with multiple myeloma: 3 patients at stage IIA and 13 patients at stage IIIA, according to the criteria of Durie and Salmon *[8]*. At regular 4-week intervals the patients received the MivP chemotherapy schedule (*m*elphalan *i*ntrav*e*nously on day 1, *p*rednisone orally on days 1–4) plus 60 mg of Aredia® (pamidronate; intravenously on day 1) for inhibition of bone resorption. At the time of sample collection, the patients had received an average of 20 (range 11–30) MivP courses and an average of 9 (range 1–17) pamidronate courses.

The patients in Groups C and D (tumor patients without bone metastases; tumor patients with bone metastases; and multiple myeloma patients) did not significantly differ among each other with respect to chronological age ($P > 0.05$).

All serum specimens were taken between 0800 and 0900 h. Urine samples (second morning samples) were obtained between 0800 and 1000 h. Sera were separated from the blood clot within 4 h after specimen collection and stored at −20 °C until analysis. There were no repeated freeze–thaw cycles in any of the samples analyzed. All procedures concerning human subjects were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

ASSAYS

Determination of bone sialoprotein in serum. The serum concentrations of human bone sialoprotein were determined by an RIA (Immundiagnostik, Bensheim, Germany; no. K4221) that utilizes a polyclonal antibody raised in chicken against purified human bone sialoprotein. Briefly, 100 μL of serum was mixed with 100 μL of this antibody solution and ^{125}I-labeled bone sialoprotein (100 μL). After an incubation step of 24 h at 4 °C, 100 μL of second-antibody solution (raised in donkeys against chicken IgG) was added. After another incubation step of 2 h at 4 °C, the reaction mixture (containing antibody-bound radioactivity) was centrifuged for 10 min at 2000*g*, and the supernatant was aspirated. After a washing step (with 250 μL of an aqueous solution of 9 g/L NaCl and 60 g/L polyethylene glycol), another centrifugation step (10 min, 2000 g) was performed and the supernatant was again aspirated. Radioactivity in the pellet was measured for 1 min with a gamma-counter.

A calibration curve was constructed by use of a four-parameter curve-fitting algorithm. Intraassay imprecision (CV) was 5.3% (mean = 9.8 μg/L; n = 20); between-assay imprecision was 9.1% (mean = 11.1 μg/L; n = 20). The lower limit of detection (determined by 20 replicate analyses of the zero calibrator and calculation of the concentration that corresponds to the 95th percentile of the counts obtained) was 0.7 μg/L. We used calibrators with the following assigned values: 0, 1.9, 3.8, 7.5, 15, 30, 60, and 120 μg/L. Assessment of analytical accuracy by adding calibrator to patients' samples yielded a mean recovery rate of 99.4%. All analyses were done in duplicate within a single run.

Determination of bone alkaline phosphatase mass concentration in serum. We determined bone alkaline phosphatase mass concentration with an IRMA (Tandem®-R Ostase™, no. 3040 BE; Hybritech, San Diego, CA), which uses two monoclonal antibodies against two different epitopes of the bone alkaline phosphatase molecule. A calibration curve was constructed by linear interpolation between the plotted analytical results. The following reference intervals (2.5th–97.5th percentiles) for bone alkaline phosphatase mass concentration in serum were established in apparently healthy volunteers from the staff of our hospital: 3.8–21.3 μg/L for men (n = 50) and 3.4–15.0 μg/L for women (n = 50), ages 20–55 and 18–56 years, respectively. Interassay CVs were 4–12% for the whole range of measured bone alkaline phosphatase concentrations *[9]*.

Determination of the carboxyl-terminal propeptide of procollagen type I in serum. The concentration of the carboxyl-terminal propeptide of procollagen type I in serum was determined by an enzyme immunoassay (Prolagen-C™; Metra Biosystems, Palo Alto, CA). A calibration curve was constructed by a four-parameter curve-fitting algorithm. The following reference intervals (2.5th–97.5th percentiles) for the carboxyl-terminal propeptide in serum were established in apparently healthy persons: 50–180 μg/L for men, ages 23–58 years (n = 51), and 50–145 μg/L for women, ages 23–59 years (n = 51). Interassay CVs were

7–10% for the whole range of measured carboxyl-terminal propeptide concentrations *[9]*.

Determination of the urinary excretion of deoxypyridinoline. Urinary excretion of deoxypyridinoline was determined by a competitive enzyme immunoassay (Pyrilinks-D™; Metra Biosystems) based on the use of a monoclonal antibody against deoxypyridinoline. This assay measures the free (i.e., not peptide-bound) fraction of deoxypyridinoline. A calibration curve was constructed by using a four-parameter curve-fitting equation. Urinary excretion was normalized for creatinine excretion. The following reference interval (2.5th–97.5th percentiles) for excretion of deoxypyridinoline in second morning urine samples (obtained between 0800 and 1000 h) from the apparently healthy persons (n = 102; 51 men and 51 women, ages 19–62 years): 1.3–9.3 μmol/mol creatinine. There was no dependence of reference values on sex ($P > 0.1$). Between-assay imprecision was 8–12% for the whole range of measured deoxypyridinoline concentrations.

Determination of the urinary excretion of calcium. Calcium excretion in second morning urine samples (see above) was determined with an EFOX 5053 flame emission spectrometer (Eppendorf Gerätebau Netheler + Hinz, Hamburg, Germany). Data are given as mol/mol creatinine (upper reference limit: 0.6 mol/mol creatinine).

Determination of serum creatinine concentrations and γ-glutamyltransferase activity. Serum creatinine concentrations were determined with a kinetic modification of the Jaffe procedure *[10]*; upper reference limits were 115 μmol/L for men and 97 μmol/L for women. Serum γ-glutamyltransferase activity (EC 2.3.2.2) was determined according to Szász *[11]* (upper reference limit: 28 U/L for men and 18 U/L for women).

STATISTICAL ANALYSIS

All values are given as mean ± SD. The statistical methods used include the *U*-test according to Wilcoxon and Mann and Whitney (two-tailed) for unpaired variables, and Wilcoxon's ranked sum test for paired variables, linear regression equations, and linear correlation coefficients. To examine whether the values were gaussian distributed in apparently healthy persons, we performed the Kolmogorov–Smirnov test. Kruskall–Wallis one-way analysis of variance was performed for assessing a putative association between kidney and liver dysfunction on the one hand and serum bone sialoprotein concentrations on the other. A serum γ-glutamyltransferase activity/creatinine concentration above the upper reference limit (see above) was considered an indicator of liver/kidney dysfunction. In patients with bone metastases and multiple myeloma, z-scores were calculated according to the formula $(x_i - M)/SD$, where x_i is the value for an individual patient, and M and SD are the mean and SD of the values observed in tumor patients without bone metastases. All statistical calculations were performed with the aid of SPSS/PC+™ V2.0 (SSPS, Chicago, IL).

RECEIVER OPERATING CHARACTERISTIC (ROC) PLOTS

To compare the diagnostic efficacy of serum bone sialoprotein and established markers of bone turnover in tumor patients, we established ROC plots as follows: (*a*) Decision thresholds were fixed that corresponded to the 3rd, 4th, . . . , 97th percentiles of the distribution of the marker values in all tumor patients examined. (*b*) Graphical presentation of the ROC curve was made by linear interpolations between the data pairs obtained by calculating the diagnostic sensitivities and specificities at these threshold values. (*c*) The area under the curve (mean ± SE) was calculated by an algorithm from Hanley and McNeil *[12]* in which the marker values (x) were classed into the following groups: x <3rd percentile, 3rd percentile ≤ x < 4th percentile, . . . , 96th percentile ≤ x < 97th percentile, x ≥97th percentile (see above). (*d*) For evaluating statistically significant differences in the areas under different ROC curves, we used a technique provided by Hanley and McNeil *[13]*. The significance level was set at $P = 0.05$. All calculations were made by means of a program written by one of us (W.W.) with use of SPSS/PC+ V2.0 and implementing the algorithms mentioned above.

Results

SERUM BONE SIALOPROTEIN CONCENTRATIONS

In apparently healthy persons. Serum bone sialoprotein concentrations in apparently healthy adults (Group A) did not differ with respect to sex ($P > 0.1$). Therefore, we determined the reference interval (2.5th–97.5th percentiles) for all 68 apparently healthy persons examined. The results, 2–23 μg/L (median 12 μg/L), showed a gaussian distribution.

In postmenopausal women (Group B), serum bone sialoprotein concentrations (27.3 ± 11.9 μg/L) increased by an average 142% over the concentrations in premenopausal women ($P < 0.001$). Serum bone sialoprotein values were also greater in older men (Group B men), 18.4 ± 5.0 μg/L, than in younger men, the average increase being 63% ($P < 0.001$) (Fig. 1).

INFLUENCE OF LIVER AND KIDNEY DYSFUNCTION ON THE BONE SIALOPROTEIN VALUES

In patients with liver and kidney dysfunction. In patients with various malignant tumors showing either the presence or the absence of bone metastases by bone scintigraphy, serum creatinine concentrations ranged from 60 to 135 μmol/L (median 90 μmol/L) and γ-glutamyltransferase activity ranged from 6 to 250 U/L (median 17 U/L). There was no significant influence of liver and kidney dysfunction (as defined in *Materials and Methods*) on serum bone sialoprotein values ($P > 0.1$).

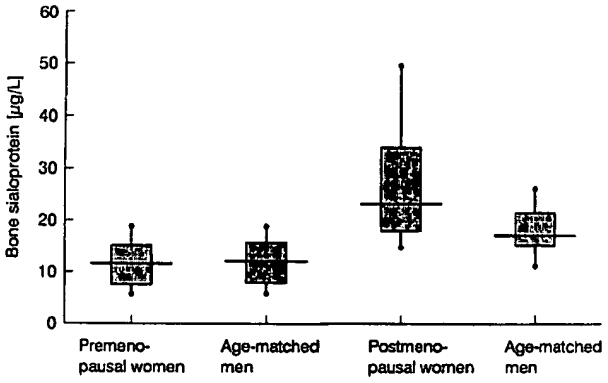

Fig. 1. Comparison of serum bone sialoprotein values in pre- and postmenopausal women and age-matched men.

Upper and lower lines indicate the 10th and 90th percentiles, *boxes* the 25th and 75th percentiles, and the *line in the box* the 50th percentile of the values.

CLINICAL USEFULNESS IN PATIENTS WITH METASTATIC SPREAD INTO BONE

Figure 2 (panels a-c) compares concentrations of serum bone sialoprotein with those of established marker substances of bone turnover. For further statistical analysis, three outliers (indicated by arrows in Fig. 2, a-c) were eliminated.

In patients with bone metastases (according to scintigraphic criteria) serum bone sialoprotein concentrations were greater ($P < 0.05$) than in tumor patients without metastatic spread into bone. The same applied to serum bone alkaline phosphatase ($P < 0.001$), carboxyl-terminal propeptide values ($P < 0.001$), and the urinary excretion of deoxypyridinoline ($P < 0.001$) (Table 1). In tumor patients without bone metastases, no significant difference was found in serum bone sialoprotein concentrations between patients with breast cancer (24 ± 8 µg/L) and those with other cancer types (22 ± 6 µg/L) ($P > 0.05$).

Comparison of tumor patients with bone metastases and those without bone metastases by z-score analysis showed significantly lower z-scores for serum bone sialoprotein than for serum bone alkaline phosphatase ($P < 0.001$). The same applied to the comparison with the z-score values of serum carboxyl-terminal propeptide and urinary deoxypyridinoline, although this difference did not attain statistical significance ($P > 0.05$) (Fig. 3).

Tumor patients with bone metastases were also compared with those without bone metastases by ROC analysis. The area under the curve was lower for serum bone sialoprotein than for all other bone turnover markers; however, only for the comparison with serum bone alkaline phosphatase did the difference attain statistical significance ($P < 0.05$) (Fig. 4).

In patients with bone metastases the correlation between serum bone sialoprotein concentrations and bone alkaline phosphatase/carboxyl-terminal propeptide values was statistically significant, albeit weak ($P < 0.01$). We observed no correlation ($P > 0.05$) between serum bone sialoprotein and the urinary excretion of deoxypyridinoline (Table 2 and Fig. 2, d-f).

CLINICAL USEFULNESS IN MULTIPLE MYELOMA PATIENTS

In multiple myeloma patients, serum bone sialoprotein concentrations were significantly less than in tumor patients without bone metastases ($P < 0.001$). The same was true for serum bone alkaline phosphatase ($P < 0.001$) and serum carboxyl-terminal propeptide ($P < 0.001$) but not for urinary deoxypyridinoline ($P > 0.05$) (Table 1).

Comparison of multiple myeloma patients and tumor patients without bone metastases by z-score analysis revealed no significant differences between the z-score values for serum bone sialoprotein and for serum bone alkaline phosphatase/carboxyl-terminal propeptide ($P > 0.05$) (Fig. 3).

Serum bone sialoprotein concentrations were not significantly correlated with the values of the other biochemical marker substances examined ($P > 0.5$), but serum bone alkaline phosphatase/carboxyl-terminal propeptide values were correlated with urinary deoxypyridinoline (Table 2). There was a correlation between serum bone sialoprotein concentrations and the number of pamidronate courses applied ($r = -0.578$, $P < 0.05$) and a slight but not significant correlation between serum bone sialoprotein and the number of MivP courses applied ($r = 0.499$, $P = 0.098$). None of the multiple myeloma patients showed serum γ-glutamyltransferase activities or creatinine concentrations above the respective upper reference limits.

Discussion

Significantly higher serum bone sialoprotein values were found in tumor patients with bone metastases than in those without bone metastases. Nothing is at present known about the extraskeletal fate of the circulating fraction of this bone matrix protein. A multivariate statistical approach revealed that neither liver nor kidney dysfunction affects its serum values. Compared with other bone matrix proteins, bone sialoprotein is relatively restricted to bone. However, bone sialoprotein immunoreactivity has also been found in several other tissues (e.g., platelets, hypertropic chondrocytes of the growing plates, and placental trophoblasts) *[1, 14]*. In an immunohistochemical study *[15]*, most of the breast carcinoma specimens examined showed a significant expression of bone sialoprotein immunoreactivity, which might in part explain the preferred homing of breast carcinoma cells to bone. In the group of tumor patients without bone metastases, we found no significant difference in serum bone sialoprotein concentrations when the breast cancer patients were compared with other cancer types.

Untreated multiple myeloma patients have low osteoblastic activity, indicating unbalanced remodeling. Osteocalcin, which is synthesized by osteoblasts and regarded as a specific marker of bone formation, is decreased in patients with excessive lytic lesions *[16, 17]*. In addition, a significant decrease of bone alkaline phosphatase has been observed during corticosteroid treatment *[18]*. Bisphosphonates can act not only on osteoclasts but also

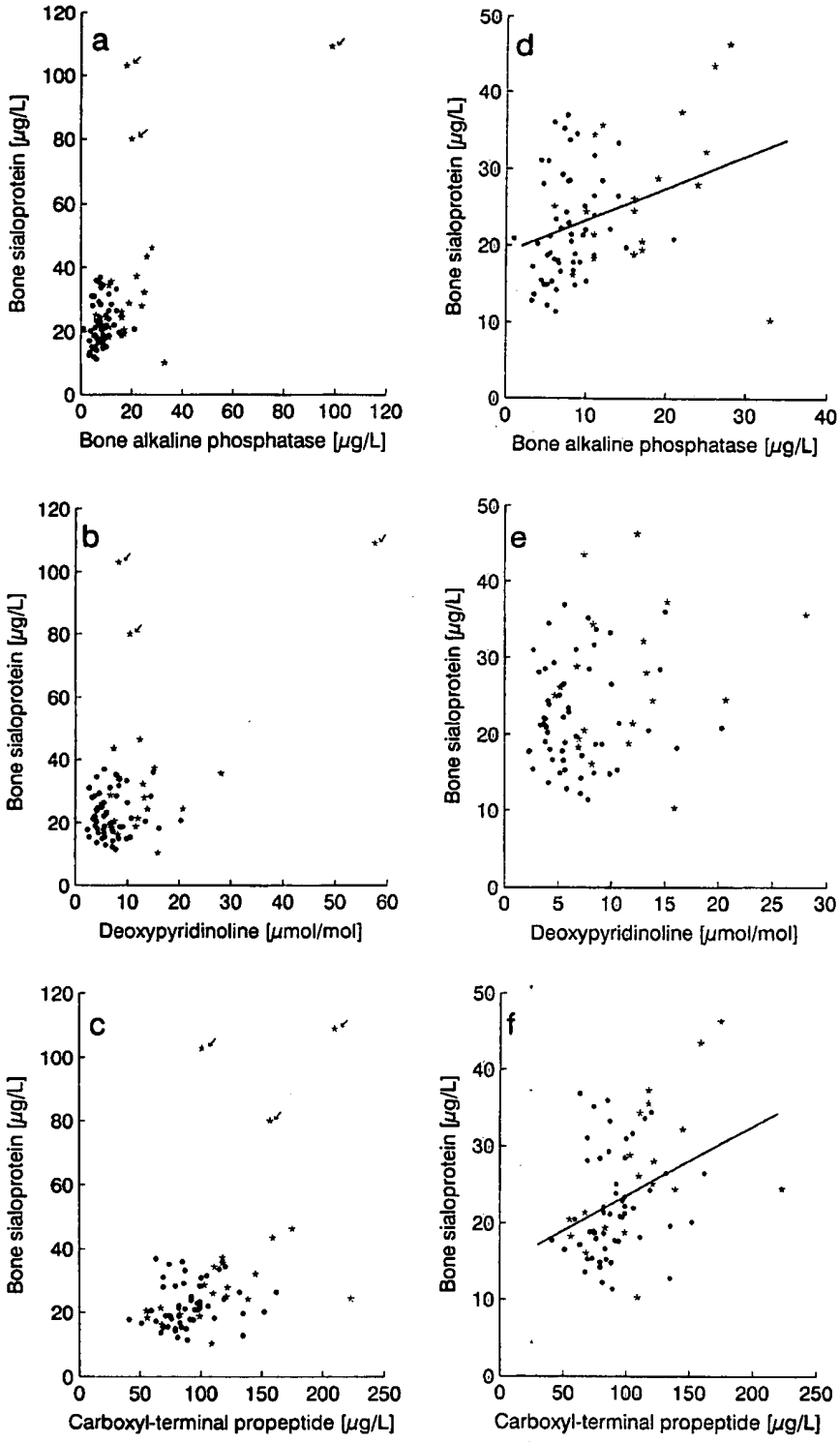

Fig. 2. Relations between serum bone sialoprotein (*y*) and established serum markers of bone turnover (*x*): bone alkaline phosphatase mass (*a* and *d*), deoxypyridinoline mass, referred to creatinine (*b* and *e*), and carboxyl-terminal propeptide mass (*c* and *f*).

Panels a–c: Values from all 77 tumor patients examined, both for sera from patients with bone metastases (*) and for sera from patients without metastatic spread into bone (●). *Panels d–f*: More detailed presentation of the data from the remaining 74 tumor patients after elimination of 3 "outlier" patients (indicated by *arrows* in *a–c*). In these 74 patients the relations between the analytes are given by the following regression equations: (*d*), $y = 0.418x + 19.09$ ($r = 0.340$; $P < 0.01$); (*e*) correlation not significant ($P = 0.06$); (*f*) $y = 0.090x + 14.52$ ($r = 0.360$; $P < 0.01$).

Table 1. Serum concentrations (mean ± SD) of bone sialoprotein in comparison with established marker substances of bone turnover in patients with malignant bone diseases.

Bone disease group	Serum conc, μg/L			Urine conc.[a]	
	Bone sialoprotein	Bone alkaline phosphatase	Carboxyterminal propeptide	Deoxypyridinoline, μmol/mol	Calcium, mol/mol
Tumors, no bone metastases	22.1 ± 6.7	7.7 ± 3.0	90 ± 24	6.6 ± 3.3	0.27 ± 0.16
Tumors, and bone metastases	26.9 ± 9.4[b]	17.6 ± 1.7[c]	119 ± 38[c]	11.7 ± 6.3[c]	0.19 ± 0.13[b]
Multiple myeloma	13.4 ± 7.5[c]	2.4 ± 1.1[c]	60 ± 19[c]	5.4 ± 1.9	Not done

[a] Normalized in terms of urinary creatinine excretion.
[b,c] Significantly different from values for tumor patients without bone metastases: [b] $P < 0.05$, [c] $P < 0.001$.

on osteoblasts *[19, 20]*; when added to cultured osteoblasts, they inhibit these cells from producing osteoclast-stimulating activity *[21]*. In patients with vertebral osteoporosis and no biochemical evidence of increased bone turnover who were then treated with oral pamidronate, serum alkaline phosphatase decreased significantly, by 20%, after 6 months of treatment *[22]*.

In tumor patients with bone metastases, we found a statistically significant, albeit weak, correlation between serum bone sialoprotein and bone formation markers, whereas no correlation was observed between serum bone sialoprotein and the urinary excretion of deoxypyridinoline. Therefore, we suggest that bone sialoprotein should be judged as a putative marker of bone formation rather than resorption. In addition, in tumor patients with bone metastases, serum bone sialoprotein seems to be inferior to all established markers of bone turnover, with respect to z-score and ROC curve analysis. Recently, Demers et al. *[23]* found that biochemical markers of bone turnover differ with respect to the efficacy by which they predict the presence of bone metastases.

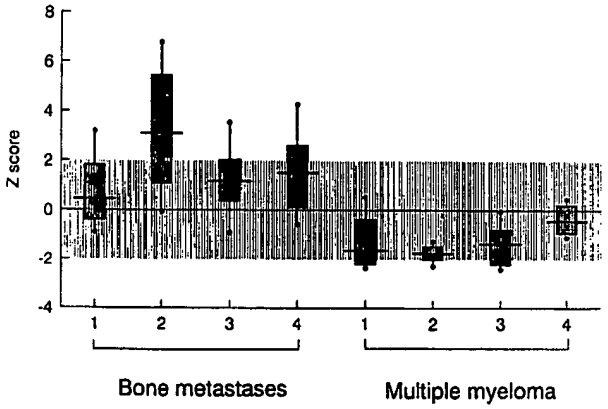

Fig. 3. Serum bone sialoprotein (*1*), serum bone alkaline phosphatase (*2*), serum carboxyl-terminal propeptide (*3*), and urinary deoxypyridinoline (*4*) in tumor patients with metastatic spread into bone and in multiple myeloma patients.

Upper and lower lines indicate the 10th and 90th percentiles, *boxes* the 25th and 75th percentiles, and *the line in the box* the 50th percentile of the z-score values, calculated as described in the text. *Shaded area*: mean ± 2 SD of the values observed in tumor patients without bone metastases.

We could discern no sex-related difference in the bone sialoprotein values in apparently healthy younger persons, whereas bone alkaline phosphatase mass *[24]* and activity concentrations *[25]* have been reported as significantly higher in men than in women, possibly reflecting a suppressive effect of estrogens on bone metabolism in women *[24]*. To our knowledge, the regulatory role of estrogens in bone sialoprotein synthesis is at present unknown. In postmenopausal women, a marked increase of bone sialoprotein values was found as compared with the concentrations in the premenopausal reference collective. The same applied to older men in comparison with

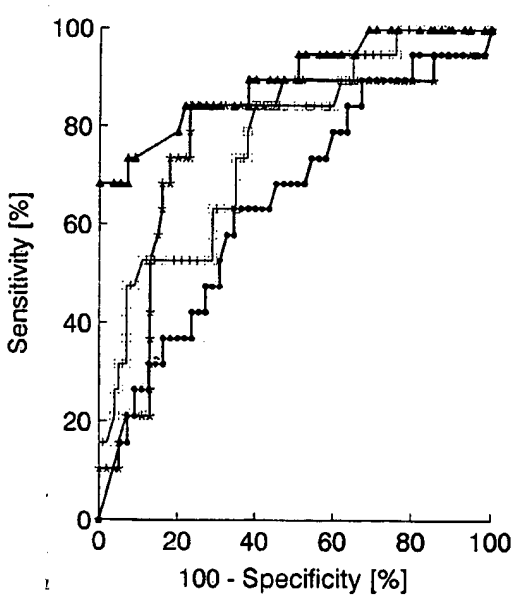

Fig. 4. ROC curves for discriminating between tumor patients with and those without bone metastases (as revealed by bone scintigraphy).

The variables examined are serum bone sialoprotein (●), serum bone alkaline phosphatase (▲), serum carboxyl-terminal propeptide of procollagen type I (*), and urinary deoxypyridinoline (□). The respective areas under the curve (mean ± SE) were 0.661 ± 0.074, 0.897 ± 0.047, 0.764 ± 0.086, and 0.797 ± 0.044. The areas for bone sialoprotein vs bone alkaline phosphatase, for bone alkaline phosphatase vs carboxyl-terminal propeptide, and for bone alkaline phosphatase vs deoxypyridinoline differed significantly from each other ($P < 0.05$). Those for bone sialoprotein vs carboxyl-terminal propeptide, for bone sialoprotein vs deoxypyridinoline, and for deoxypyridinoline vs carboxyl-terminal propeptide did not ($P > 0.05$).

Table 2. Correlation coefficients for the relations between serum bone sialoprotein and established marker substances of bone turnover in different groups of tumor patients.[a]

Patient group	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Tumors, no bone metastases	0.243	0.124	0.138	0.192	0.153	−0.040
Tumors and bone metastases	0.340[c]	0.360[c]	0.219	0.424[d]	0.455[d]	0.310[c]
Multiple myeloma	−0.206	0.046	0.060	0.218	0.583[b]	0.628[b]

[a] A, bone sialoprotein; B, bone alkaline phosphatase; C, carboxyl-terminal propeptide; D, urinary deoxypyridinoline.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

younger men. This increase corresponds to the well-known phenomenon of an age- and menopause-associated increase in bone turnover markers *[26, 27]*. Further studies should therefore be done to elucidate whether serum bone sialoprotein is useful for predicting high bone turnover after the menopause.

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